The Potential Role of Ectomycorrhizal Fungi in Determining Douglas-fir Resistance to Defoliation by the Western Spruce Budworm (Lepidoptera: Tortricidae)

BARBARA L. PALERMO[1], KAREN M. CLANCY[2], AND GEORGE W. KOCH[1]

J. Econ. Entomol. 96(3): 783–791 (2003)

ABSTRACT There is phenotypic variation among individual trees of interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) in their resistance to defoliation by the western spruce budworm (*Choristoneura occidentalis* Freeman). We evaluated the potential role of ectomycorrhizal fungi in determining this resistance using half-sib seedlings derived from parent trees that are resistant versus susceptible to budworm defoliation in the field. The seedlings were inoculated with *Laccaria bicolor* ectomycorrhizal fungi, fertilized, or untreated. Approximately 48 d after treatment, late-instar larvae from a nondiapausing laboratory colony of *C. occidentalis* were allowed to feed on pairs of resistant versus susceptible seedlings for 1 wk. Chemical analyses of current-year shoots for nitrogen (N), phosphorus (P), magnesium (Mg), and zinc (Zn) indicated that the fungus increased foliar concentrations of P and Mg in resistant seedlings, but it did not increase their growth rate. However, *L. bicolor* had no effect on foliar concentrations of P or Mg in susceptible seedlings, even though seedling growth rates increased slightly in response to the inoculation. *L. bicolor* had no effect on foliar levels of N or Zn in any of the seedlings. As expected, fertilization increased levels of N and P in the foliage of both resistant and susceptible seedlings, but it did not affect levels of Mg and Zn. Surprisingly, the fertilizer treatment had no effect on seedling growth rates. Despite these differences, late-instar budworms showed no feeding preference among untreated, mycorrhizal, or fertilized seedlings. The fact that seedlings from resistant versus susceptible Douglas-firs responded differently to the *L. bicolor* treatment lends preliminary support to the hypothesis that ecotmycorrhizae might play a role in Douglas-fir resistance to damage from the western spruce budworm. Finally, differences in foliar concentrations of N and P among untreated seedlings from different maternal trees suggested that foliar nutritional chemistry is influenced by the tree's genotype.

KEY WORDS *Choristoneura occidentalis*, fertilization, foliar nutrients, *Laccaria bicolor*, larval feeding preferences, minerals

MYCORRHIZA, THE SYMBIOTIC ASSOCIATION between plant roots and soil fungi, plays a potentially important role in regulating the interaction of trees with forest insects. Most studies of mycorrhiza-herbivore interactions have examined how aboveground herbivory affects mycorrhizae, whereas few have examined how mycorrhizae affect tree resistance to herbivores (Gehring and Whitham 2002). Mycorrhizae are ubiquitous among coniferous forest trees, including Douglas-fir (Miller 1982, Fogel and Hunt 1983). The external hyphae of ectomycorrhizal fungi act to expand the root system of infected plants, allowing them to acquire resources beyond the rhizosphere, increasing levels of many macro and micronutrients in shoot tissues and enhancing plant growth (Marschner and Dell 1994, Yanai et al. 1996, Schowalter et al. 1997).

Mycorrhizal associations may affect plant resistance to herbivory by several mechanisms that are founded on the ability of the fungal symbiont to improve host plant resource acquisition, including water and/or mineral nutrients (Gehring et al. 1997). By increasing host plant vigor, mycorrhizae could improve performance of herbivores that prefer vigorous host plants (Price 1991). Conversely, for systems wherein herbivore performance is better on less vigorous plants (White 1984, Waring and Cobb 1992), mycorrhizae may improve plant resistance by increasing plant vigor. Mycorrhizae might also alter plant production of antiherbivore defensive compounds by changing levels of mineral elements required for biosynthesis (Jones and Last 1991). Finally, mycorrhizae might alter tree resistance by changing the nutritive value of plant tissue, including levels of nitrogen (N), phosphorus (P), and other elements, making tissues either more or less nutritious to herbivores.

The important influence of mycorrhizae on plant growth and nutrient levels in plant tissues is the basis

[1] Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011–5640.

[2] USDA Forest Service Research and Development, Rocky Mountain Research Station, Southwest Forest Science Complex, 2500 S. Pine Knoll Drive, Flagstaff, AZ 86001–6381.

for expecting that they might affect plant resistance to herbivores such as the western spruce budworm (*Choristoneura occidentalis* Freeman). For example, interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) trees that are phenotypically resistant to damage from *C. occidentalis* had higher levels of N and sugars, plus lower mineral/N ratios for P/N, magnesium (Mg)/N, potassium (K)/N, and zinc (Zn)/N, in their foliage than susceptible trees (Clancy 2001). Resistant trees also had greater radial growth rates compared with susceptible trees in two of three populations studied (Clancy 2002). However, foliar concentrations of terpenes were not different between resistant and susceptible trees, suggesting that terpenes are probably not important defensive compounds for the western spruce budworm (Clancy 2001, 2002; Chen et al. 2002b).

Four mechanisms of interior Douglas-fir resistance to damage from the western spruce budworm have been documented (Clancy 2002; K.M.C., Zhong Chen, and Thomas E. Kolb [Northern Arizona University] unpublished data); three of the mechanisms could be linked with potential mycorrhizal mediation of herbivore resistance. First, phenological asynchronony (Clancy et al. 1993; Chen et al. 2001a, 2003; Clancy 2002) resulting from delayed bud burst enables resistant trees to reduce the exposure of vulnerable developing tissues to western spruce budworm larvae when they emerge to feed on swollen buds in the spring; this seems unlikely to be influenced by mycorrhizae. Second, foliar nutritive quality influences budworm larval performance, with intermediate levels of sugars and key elements including P, Mg, and K (plus mineral/N ratios) being optimal and lower and higher tissue concentrations reducing larval performance (Clancy 1992a, 1992b, 2001, 2002; Clancy and King 1993). Variation among trees in foliar nutrients could be associated with mycorrhizae. Third, shoot vigor, measured as growth rate, is positively related to western spruce budworm resistance (Clancy et al. 1993; Chen et al. 2001a, 2002a), and tree growth rates could also be affected by mycorrhizae. The fourth mechanism is induced susceptibility, whereby defoliation alters foliar nutrients to make trees more favorable for insect feeding (K.M.C., Zhong Chen, and Thomas E. Kolb [Northern Arizona University] unpublished data). Differences in mycorrhizae might help explain why susceptible trees appear to be more prone to changes in foliar nutritional chemistry in response to western spruce budworm defoliation than resistant trees.

The objective of this study was to test the hypothesis that ecotmycorrhizae increase the resistance of Douglas-fir trees to the western spruce budworm by increasing levels of foliar nutrients beyond the range that is optimal for budworm performance. We used half-sib seedlings grown from open-pollinated cones collected from resistant and susceptible Douglas-firs (Chen et al. 2001a) to address the following questions: (1) Do ectomycorrhizal fungi change the chemical composition of Douglas-fir foliage? (2) Do budworm larvae respond to ectomycorrhizal-induced changes via feeding choice behavior? (3) What is the potential role of this relationship in determining host-plant resistance to defoliation by the budworm? We included fertilized seedlings to ensure the comparison of different levels of foliar nutrients in the event of unsuccessful inoculation with ecotmycorrhizae, and to examine the role of altered foliar nutrition apart from other potential influences of mycorrhizae. We also examined variation in levels of foliar N, P, Mg, and Zn among untreated (i.e., control) seedlings from different maternal trees to determine if there might be a heritable genetic basis for Douglas-fir nutritional chemistry.

Materials and Methods

Western Spruce Budworms. We used *C. occidentalis* larvae from a nondiapausing laboratory culture maintained at the Rocky Mountain Research Station in Flagstaff, AZ. The insects from this laboratory colony do not differ in performance from natural budworm populations (Leyva et al. 1995).

Douglas-fir Seedlings. We used 30–90-cm, 3-yr-old Douglas-fir half-sib seedlings. The seedlings were grown from open-pollinated seeds collected from eight pairs of trees that are phenotypically susceptible (showing obvious signs of a history of defoliation) or resistant (healthy-looking) to the budworm on the Pike National Forest near Deckers, CO and from three pairs of trees on the San Isabel National Forest near Buena Vista, CO (Clancy 1991, 2001; Clancy et al. 1993). The resistant and susceptible parent trees were paired in the field (within 60 m of one another) based on similarities in age, height, and microsite. Seeds were collected from the 11 pairs of mature Douglas-fir trees (11 resistant trees plus 11 susceptible trees, for a total of 22 parent tree genotypes) to produce the half-sib seedlings, which were grown in the Rocky Mountain Research Station greenhouses in Flagstaff, AZ (Chen et al. 2001a). We matched half-sib seedlings derived from the original pairs of resistant and susceptible parent trees throughout the experiments to control for variation among the 11 pairs. The seedlings were raised in a nutrient-poor peat moss-vermiculite growing medium and had not been fertilized for the previous 2 yr.

The seedlings were subjected to one of three treatments: 36 were inoculated with ectomycorrhizal fungi, 36 were fertilized, and 108 served as untreated controls. Seedlings from all three groups were removed from their small plastic pots (15 cm in diameter $\times$ 20 cm in height) and transplanted into larger pots (30 cm in diameter $\times$ 27 cm in height). All of the seedlings were irrigated with pH-adjusted water to promote fungal growth. Soil pH was checked regularly for all treatments to ensure that it remained between 5.0 and 6.0. Most species of mycorrhizal fungi prefer slightly acidic soils; pH levels that are too high or too low can kill the fungus and inhibit the uptake of nutrients (Marschner and Dell 1994, Edmonds et al. 2000).

Inoculation with Ectomycorrhizal Fungi. At the time of transplant, 36 seedlings (18 resistant and 18

Table 1. Distribution among the five treatment comparisons of 79 pairs of half-sib seedlings grown from open-pollinated seed collected from 11 pairs of mature Douglas-fir trees that were resistant versus susceptible to western spruce budworm damage

Treatment comparison[a]	Pair no. for maternal parent trees											Total no. pairs
	1	2	3	4	5	6	7	8	9	10	11	
1. Res. control vs. Sus. control	2	1	2	1	1	1	1	1	1	1	–	12
2. Res. control vs. Sus. inoculated	4	2	2	2	–	1	1	1	1	–	2	16
3. Res. inoculated vs. Sus. control	3	2	2	2	1	1	1	1	1	1	2	17
4. Res. control vs. Sus. fertilized	3	1	2	2	1	1	1	1	1	1	2	16
5. Res. fertilized vs. Sus. control	4	2	2	2	1	1	1	1	1	1	2	18
Total no. pairs	16	8	10	9	4	5	5	5	5	4	8	79

[a] Control seedlings were untreated; inoculated seedlings were treated with *Laccaria bicolor* ectomycorrhizal fungi; fertilized seedlings were treated with a 15 N:30 P:15 K water soluble fertilizer.

susceptible) were artificially inoculated with *Laccaria bicolor*, an ectomycorrhizal fungus associated with Douglas-fir trees and commonly used in nurseries (Castellano and Trappe 1985). Root samples (20 cm in length) were cut from 10 randomly selected trees (five resistant and five susceptible) and inspected under a microscope to calculate the percentage of short roots infected with the fungus before inoculation (number of infected root tips ÷ the total number of root tips). This is the same procedure used by Gehring and Whitham (1994) to compare the proportion of ectomycorrhizal colonization on resistant versus susceptible pinyon pine (*Pinus edulis* Engelmann) trees. Pretreatment inspection of the roots revealed the presence of little or no mycorrhizae (0–6%), most likely because of the highly alkaline water used for irrigation. The proportion of short roots that were infected with the fungus averaged 2 ± 0.6% (± SE, here and throughout) on both resistant and susceptible genotypes. The seedlings were root-dipped into a hydrogel containing *L. bicolor* inoculant (vegetative mycelium) grown in sterile culture on a vermiculite carrier. The inoculant (MycorTree *Laccaria*) was purchased from Plant Health Care, Inc., in Pittsburgh, PA. An absorptive compound (Terra-Sorb Fine, Plant Health Care, Inc., Pittsburgh, PA) was added to the mixture to ensure adhesion to the roots.

Fertilization. Thirty-six seedlings (18 resistant and 18 susceptible) were treated with a water-soluble fertilizer containing 15% N, 30% P, 15% K, and some essential micronutrients (0.02% boron, 0.07% copper, 0.15% iron, 0.05% manganese, and 0.06% Zn). The fertilizer was applied a total of three times (every other week) throughout the treatment period. Resistant and susceptible trees were hand-watered from a bucket containing ≈19 liters of pH-adjusted water mixed with 0.15 liters of fertilizer.

Controls. The remaining 108 seedlings (54 resistant and 54 susceptible) were untreated. The large number of controls was necessary to ensure enough were available for five sets of budworm-feeding comparisons.

Experimental Design. All seedlings were maintained in a greenhouse where temperatures were controlled by a computer to simulate early spring conditions in a high elevation Douglas-fir habitat (12 h at 4°C and 12 h at 10°C). The cooler temperatures were necessary to delay flushing and provide sufficient time for treatments (inoculation and fertilization) to have an effect on foliar nutrient content. Once individual trees began to flush, greenhouse temperatures were increased (12 h at 10°C and 12 h at 25°C) to promote flushing of the remaining trees.

We used five paired treatment comparisons (i.e., five paired *t*-tests) to evaluate the effects of parent tree category (resistant versus susceptible), ectomycorrhizae (inoculated versus not inoculated) and fertilization (fertilized versus not fertilized) on foliar nutritional chemistry, seedling growth, and budworm larval feeding choices (measured as the proportion of new needles consumed). Resistant versus susceptible half-sib seedlings were matched according to physical similarities (i.e., height, foliage density, and general architecture) to minimize potential budworm feeding preferences based on differences in host plant morphology. Originally, there were 18 pairs of resistant versus susceptible half-sib seedlings in each of the five paired *t*-tests, totaling 90 pairs (180 seedlings). However, 11 pairs had to be eliminated because one or both seedlings within the pair died during the experiment, leaving a total of 79 pairs (158 seedlings).

We compared (1) twelve pairs of resistant versus susceptible control seedlings to find out if there were inherent differences in responses (i.e., foliar nutritional chemistry, seedling growth, and larval feeding preferences) between seedlings from the two parent tree categories, (2) sixteen pairs of resistant control versus inoculated susceptible seedlings to determine whether ecotmycorrhizae changed responses for susceptible seedlings, (3) seventeen pairs of resistant inoculated versus susceptible control seedlings to decide whether ecotmycorrhizae changed responses for resistant seedlings, (4) sixteen pairs of resistant control versus susceptible fertilized seedlings to find out whether fertilization changed responses for susceptible seedlings, and (5) eighteen pairs of resistant fertilized versus susceptible control seedlings to determine whether fertilization changed responses for resistant seedlings. Pairs of resistant versus susceptible seedlings from the 11 different maternal tree pairs were distributed in approximately even proportions among the five treatment comparisons to avoid any bias (Table 1).

Chemical Analyses. Three or four newly flushed shoots were sampled from each seedling. The Ana-

lytical Services Laboratory at Northern Arizona University analyzed foliage samples for total Kjeldahl N and P (colorimetrically), plus Mg and Zn (by flame atomic absorption spectroscopy). These four key nutrients are known to affect budworm performance (Clancy 1992a, 1992b, 2001, 2002; Clancy and King 1993). Only the current-year shoots were sampled because nutrients accumulate in the youngest needles and because they are the preferred food source of the budworm (Fellin and Dewey 1986). Results from the analyses were used to compare foliar nutritional quality among inoculated, fertilized, and untreated seedlings. Sampling immediately preceded a larval feeding bioassay so that preferences, if any, could be linked to the nutrient content of the foliage. Trees were sampled as soon as they began to flush to preserve the chemical composition of the expanding needles. The samples were temporarily stored in zip-lock bags and frozen at 0°C until all of the trees had flushed. One foliage sample from a resistant seedling in comparison number four was missing, so there were only 15 pairs available for the foliar chemistry paired *t*-tests for that particular comparison.

Larval Feeding Bioassay. Feeding choice tests were conducted to determine which type of seedling the western spruce budworm larvae preferred in each of the five comparisons. Paired resistant and susceptible seedlings were positioned next to one another with their branches tied together to provide feeding larvae with equal access to both seedlings. Treatment time is the number of days between seedling transplant (i.e., inoculation with *L. bicolor* or initial fertilization) and foliage sampling plus the larval feeding bioassay. Treatment time ended when ≈50% of the current-year shoots on both seedlings in a pair had reached the brush (sixth) stage, which is the appropriate phenological stage for late-instar feeding (Shepherd 1983). Because seedlings flushed at different times (despite uniform greenhouse conditions), treatment time ranged from 38 to 65 d. Also, resistant seedlings tended to break bud later than susceptible seedlings (Clancy et al. 1993; Chen et al. 2001a, 2003; Clancy 2002). Once the majority of the new shoots on both seedlings in a pair had reached the brush stage, fourth and fifth instar western spruce budworms (one larva per five terminal buds) were placed on the interlocking branches. Large netted bags were placed over each pair of trees to confine the insects and allow irrigation. The larvae were permitted to feed for 1 wk, and then we visually inspected the current-year shoots on each seedling. The shoots were categorized as 0–25% consumed, 26–50% consumed, 51–75% consumed, or 76–100% consumed. Larval feeding preferences were based on the total proportion of the current-year foliage that was consumed.

Seedling Growth. We measured seedling height and basal diameter when the trees were transplanted into larger pots and again when they were ready for larval feeding. Stem height and basal diameter were combined to calculate an overall growth ratio ([posttreatment height · (diameter)^2] ÷ [pretreatment height · (diameter)^2]) to determine seedling growth as a result of treatment.

Statistical Analyses. We used paired *t*-tests to contrast the seedling (foliar concentrations of N, P, Mg, and Zn, and growth) and larval (feeding preference) responses between the paired resistant and susceptible seedlings used in each of the five treatment comparisons. One-way analysis of variance (ANOVA) tests were used to evaluate differences among the 11 resistant or susceptible parent tree genotypes in concentrations of the foliar nutrients we measured. Detectable differences among the offspring from different maternal trees would imply a heritable genetic basis for these traits. Because we used the same experimental units to measure multiple dependent variables, we could expect to detect differences for ≈0.3 of the six response variables from random chance ($P = 0.05$).

Results

Inoculation with Ectomycorrhizal Fungi. Posttreatment inspection of 10 randomly selected seedlings that were inoculated (five resistant and five susceptible) revealed higher levels of ectomycorrhizae (8–14%) compared with the pretreatment inspection (0–6%). Before inoculation with *L. bicolor*, both resistant and susceptible seedlings averaged 2 ± 0.6% fungal colonization on their roots. After treatment, the average proportion of infected roots on inoculated resistant seedlings was significantly higher (12 ± 0.7%) than the average proportion of infected roots on susceptible seedlings that were inoculated (9 ± 0.7%) ($t = -17.36$, $P < 0.001$, $n = 10$).

Effects of Mycorrhizal Fungi and Fertilization on the Chemical Composition of Douglas-fir Foliage. There were no significant differences in foliar concentrations of N, P, Mg, or Zn between the resistant versus susceptible control seedlings (Fig. 1A–D). This suggested that there were no inherent differences in foliar chemistry between the resistant versus susceptible seedlings.

The ectomycorrhizal inoculation did not change the foliar chemistry of the susceptible seedlings (Fig. 1A–D). However, it significantly increased foliar concentrations of P and Mg in the resistant seedlings (Fig. 1B and C), although there was no effect on levels of N or Zn (Fig. 1A and D).

As expected, the fertilizer treatment caused a significant increase in foliar N in both susceptible and resistant seedlings (Fig. 1A). Likewise, foliar P increased in response to fertilization in both susceptible and resistant seedlings (Fig. 1B). The fertilization treatment had no effect on levels of Mg or Zn in either resistant or susceptible seedlings (Fig. 1C and D).

Effects of Mycorrhizal Fungi and Fertilization on Seedling Growth and Larval Feeding Preferences. Seedling growth rates were equivalent for the resistant versus susceptible control seedlings (Fig. 2A), indicating there were no inherent differences in growth rates between the resistant and susceptible seedlings. The ectomycorrhizal inoculation caused a slight but

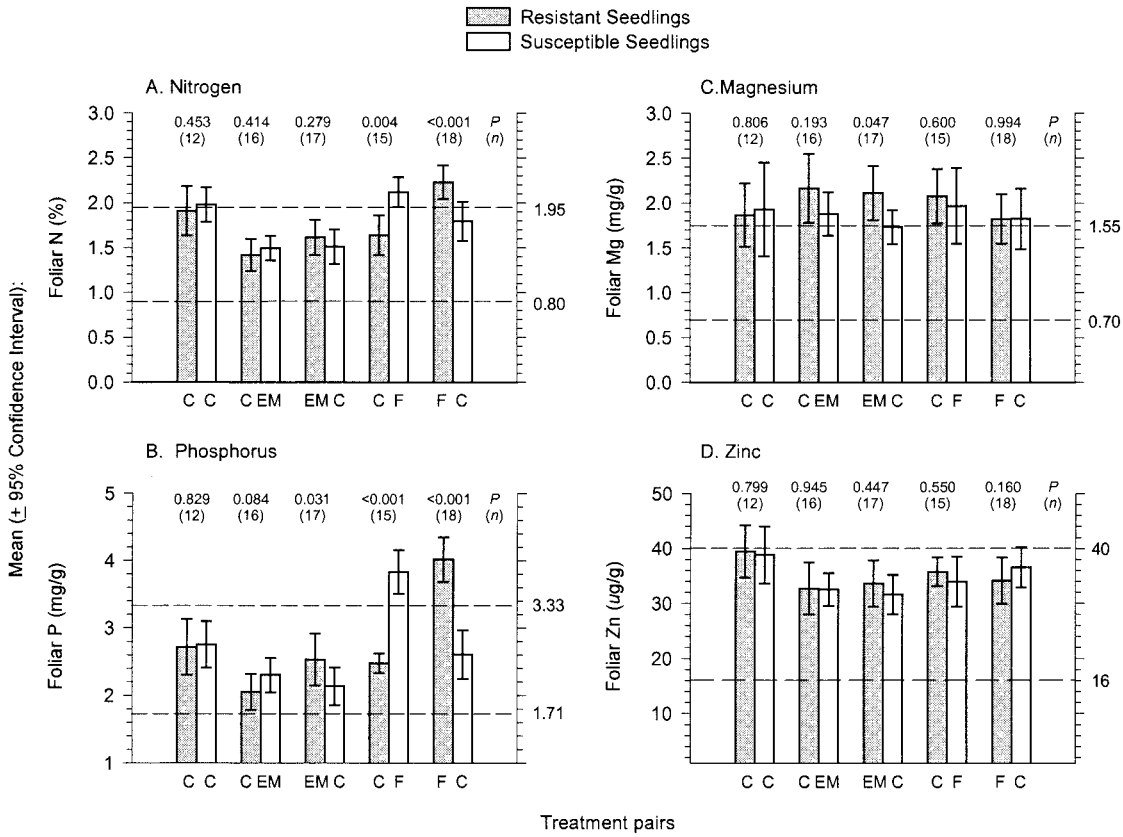

Fig. 1. Mean (±95% confidence interval) dry weight concentrations of N (A), P (B), Mg (C) and Zn (D) in the new (i.e., current-year) foliage of half-sib seedlings grown from open-pollinated seeds collected from interior Douglas-fir trees that are resistant versus susceptible to defoliation by the western spruce budworm. Results are shown for five treatment pairs, from left to right: (1) C C, resistant control (i.e., untreated) versus susceptible control seedlings; (2) C EM, resistant control versus susceptible seedlings treated with *Laccaria bicolor* ectomycorrhizal fungi; (3) EM C, resistant ectomycorrhizal-treated versus susceptible control seedlings; (4) C F, resistant control versus susceptible seedlings treated with a water-soluble 15 N:30 P:15 K fertilizer; and (5) F C, resistant fertilized versus susceptible control seedlings. The *P* values from paired *t*-tests used to compare the seedlings' responses to the two treatments in each pair are shown in the first row at the top of the figures; the second row shows the number of pairs of resistant versus susceptible seedlings used in each paired *t*-test (*n*). The dotted lines on each graph and corresponding numbers on the right y-axis show the upper and lower limits for concentrations of each nutrient reported by Clancy (2001) for 78 mature Douglas-fir trees growing in the forest; this provides reference points regarding how foliar nutrients in the greenhouse-grown seedlings compare with natural conditions in the field.

significant increase in the growth rate of the susceptible seedlings, but it had no detectable effect on growth of the resistant seedlings (Fig. 2A). Surprisingly, the fertilization treatment had no significant effect on the growth rates of either susceptible or resistant seedlings (Fig. 2A).

Our inspection of seedlings following the 1-wk larval feeding period indicated that new shoots were either in the 0–25% consumed category or in the 76–100% consumed category. Therefore, larval consumption was calculated by dividing the number of shoots in the 76–100% consumed category by the total number of suitable shoots per tree.

There were no detectable differences in western spruce budworm larval feeding preferences between the resistant versus susceptible control seedlings (Fig. 2B). Likewise, there were no significant effects on larval feeding preferences from the ectomycorrhizal or fertilization treatments (Fig. 2B).

Effects of Maternal Tree Genotype on Foliar Nutritional Chemistry of Half-sib Seedlings. Results from one-way ANOVA used to evaluate differences among the 11 resistant or susceptible parent tree genotypes in foliar concentrations of the nutrients we measured produced significant results for variation in levels of foliar N among both the resistant (Fig. 3A) and susceptible (Fig. 3B) seedlings, and for variation in concentrations of foliar P among the susceptible seedlings (Fig. 3C). These results suggested that the maternal tree genotype has significant effects on levels of foliar N and P in their offspring, which implies that foliar nutritional chemistry is under some degree of genetic control. There were no significant differences among the 11 resistant genotypes in foliar levels of P ($P =$

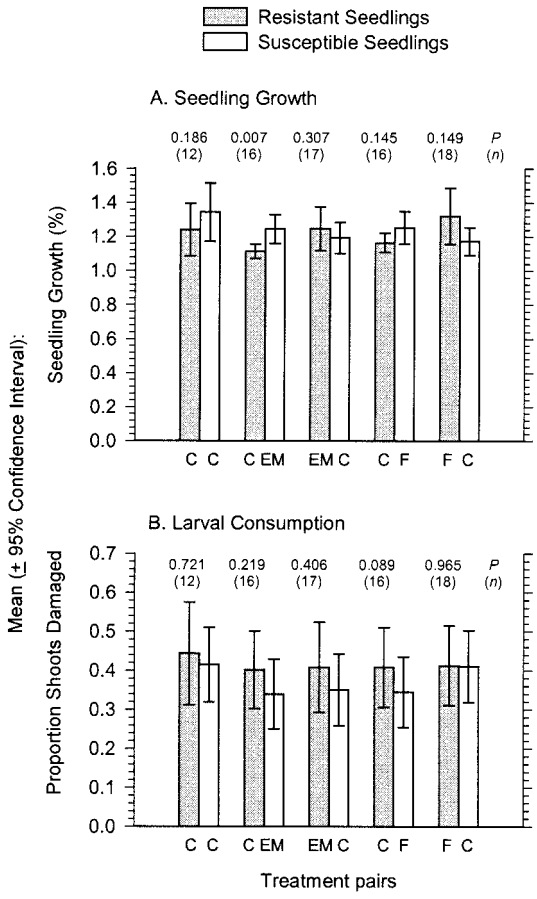

Fig. 2. Mean (±95% confidence interval) seedling growth (A) and proportion of new shoots consumed by western spruce budworm larvae (B) for half-sib seedlings grown from open-pollinated seeds collected from interior Douglas-fir trees that are resistant versus susceptible to defoliation by the western spruce budworm. For treatment pairs, *P* values and *n*, see Fig. 1.

0.091), Mg ($P = 0.419$), or Zn ($P = 0.246$) ($F_{10, 32} \leq 1.85$) or among the 11 susceptible genotypes in foliar levels of Mg ($P = 0.203$) or Zn ($P = 0.097$) ($F_{10, 36} \leq 1.80$).

Discussion

Do Ectomycorrhizal Fungi Change the Chemical Composition of Douglas-fir Foliage (or the Growth Rate of Seedlings)? *L. bicolor* increased foliar concentrations of P (Fig. 1B) and Mg (Fig. 1C) in resistant seedlings, and increased the growth of susceptible seedlings (Fig. 2A), despite the short treatment period. The effects of mycorrhizal fungi on half-sib Douglas-fir seedlings in this experiment were limited to just 48 d, on average. Yet, posttreatment inspection of roots from randomly selected seedlings revealed higher levels of fungal colonization (8–14%) compared with the pretreatment inspection (0–6%). Unusually sunny weather during the experiment caused greenhouse conditions to fluctuate, often exceeding the intended maximum temperature. As a result, the trees flushed earlier than we expected and the larval feeding bioassay (and concomitant collection of foliage samples for chemical analysis) had to be conducted earlier than planned. Nonetheless, there were detectable differences from the ectomycorrhizal inoculations on seedling growth rates and on foliar P and Mg, although the fertilizer treatment caused a much larger increase in levels of P in the resistant seedlings than the ecotmycorrhizae did (Fig. 1B). The fungus had no detectable effect on N or Zn (Fig. 1A and D).

Do Budworm Larvae Respond to Ectomycorrhizal- (or Fertilizer-) Induced Changes via Feeding Choice Behavior? Despite differences in foliar chemistry, budworm larvae showed no feeding preference among seedlings in any of the treatments (Fig. 2B). Even the dramatic increases in N and P from the fertilization treatment failed to elicit any changes in larval feeding preferences. These results suggest that late-instar budworm larvae do not distinguish between progeny from resistant versus susceptible Douglas-fir trees based on differences in growth rates or foliar chemistry. Results from larval feeding bioassays using shoots from grafted Douglas-fir clones also failed to support an important role for feeding choices made by late-instar budworms in determining resistance, at least when the foliage of resistant and susceptible Douglas-firs are at the same phenological stage (Palermo et al. 2003). Collectively, results from our larval feeding bioassays using either half-sib seedlings from resistant versus susceptible parent trees, or grafted clones of the parent trees, both support the hypothesis that budworm larvae do not discriminate.

What is the Potential Role of Ectomycorrhizal-induced Changes in the Chemical Composition of Douglas-fir Foliage (or in the Growth Rate of Seedlings) in Determining Host Plant Resistance to Defoliation by the Budworm? The inoculated half-sib seedlings from resistant parent trees had significantly more ($t_9 = -17.96, P < 0.001$) infected root tips (12 ± 0.7%) than the inoculated seedlings from susceptible parent trees did (9 ± 0.7%). The ectomycorrhizal inoculation also had different effects on the resistant versus susceptible seedlings for levels of P and Mg in the foliage (Fig. 1B and C) and for seedling growth rates (Fig. 2A). Overall, these results suggest that ectomycorrhizae might play a role in Douglas-fir resistance to damage from the western spruce budworm. The dissimilar responses to the ectomycorrhizal inoculation contrast with the similar responses the resistant and susceptible seedlings had to the fertilization treatment.

Is There Evidence for Genetic Control of Douglas-fir Foliar Nutritional Chemistry? Chen et al. (2001b) have established that there are genetically based differences between the resistant and susceptible parent trees, suggesting that the phenotypic differences observed in resistance of these interior Douglas-firs to budworm defoliation are at least partly caused by genetic differences among trees. There were detect-

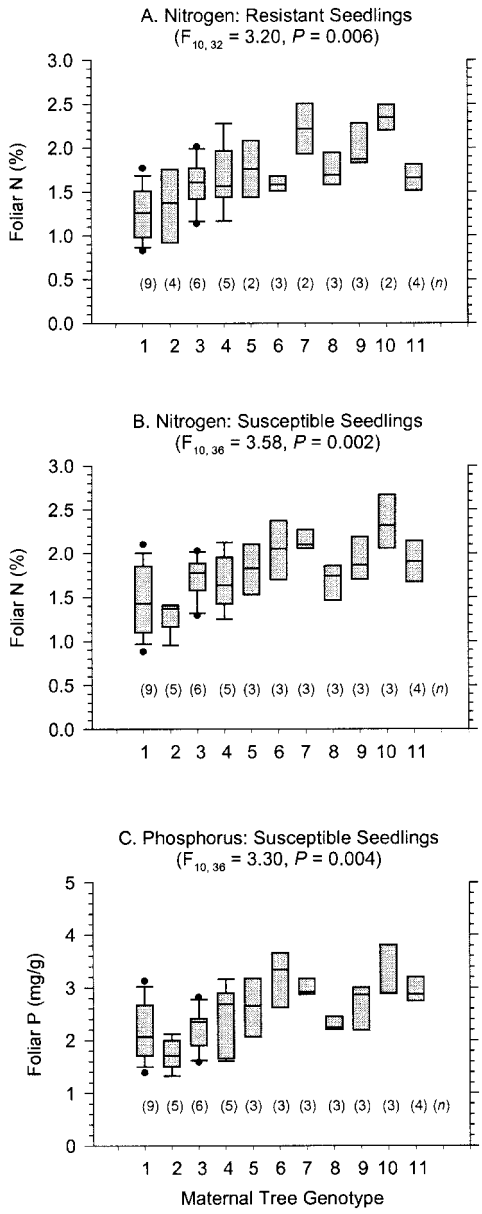

Fig. 3. Box plots comparing variation in foliar levels of N (A, B) and P (C) among 11 different maternal tree genotypes (i.e., families) of untreated (i.e., control) half-sib seedlings grown from open-pollinated seeds collected from pairs of interior Douglas-fir trees that are resistant (A) versus susceptible (B, C) to defoliation by the western spruce budworm. The F- and *P* values are from one-way ANOVAs used to evaluate the effects of maternal tree genotype on levels of the foliar nutrient; the number of seedlings in each genotype (*n*) is shown in the row at the bottom of the graphs. The lower boundary of the box indicates the 25th percentile for each genotype, the line within the box marks the median value, and the upper boundary indicates the 75th percentile. The error bars above and below the boxes for genotypes 1–4 indicate the 90th and 10th percentiles, and the dots show outlying points. The 90th and 10th percentiles are not graphed for genotypes 5–11 because the sample sizes were too small to compute that set of points.

able differences in our study among the offspring from the 11 resistant maternal genotypes in foliar concentrations of N, plus significant differences among the half-sib seedlings from the 11 susceptible maternal trees in foliar levels of N and P (Fig. 3). The existence of variation in levels of foliar nutrients among half-sib seedlings from different maternal trees suggests that foliar nutritional chemistry is influenced by the tree's genotype.

Conclusions

Muzika and Liebhold (2000) stated that despite considerable research, the effect of foliage quality on population dynamics of defoliators is not well understood. Our research suggests that budworm larvae do not distinguish between susceptible and resistant phenotypes of Douglas-fir, regardless of differences in foliar nutritional content. However, budworm defoliation may alter foliar nutrients (Van Sickle 1987; K.M.C., Zhong Chen, and Thomas E. Kolb [Northern Arizona University] unpublished data), and reduce ectomycorrhizal colonization (Kolb et al. 1999). There may also be genetically based differences in the way resistant and susceptible trees respond to mycorrhizal fungi. We must consider the results presented here as preliminary, given the short treatment period and the relatively small sample sizes. Nonetheless, they suggest that mycorrhizal fungi may affect the differences in growth and foliar chemistry observed between the resistant versus susceptible parent trees in the field.

Mycorrhizal fungi in forest ecosystems are affected by many factors including tree age and host plant genetics (Jones and Last 1991; Del Vecchio et al. 1993; Gehring and Whitham 1994, 1995). For example, young seedlings are associated with different types of mycorrhizal fungi than mature trees (Edmonds et al. 2000) and herbivore-resistant versus herbivore-susceptible trees can support very different fungal communities (Bethlenfalvay and Dakessian 1984, Gehring and Whitham 1991). Furthermore, there are >2,000 mycorrhizal species associated with Douglas-fir trees (Edmonds et al. 2000, Grove and Malajczuk 1994) that can differ in their carbon demands and ability to absorb nutrients (Jones and Last 1991). These differences can potentially affect host plant susceptibility to insect herbivores. Our results showing differences in the way the half-sib seedlings from resistant versus susceptible trees were affected by the ectomycorrhizal inoculation treatment support this possibility.

There is growing interest among forest managers in the use of ectomycorrhizal fungi in silvicultural practices. Significant gains can be achieved in terms of seedling survival, growth, and productivity, especially in stressed environments and in areas in which soil nutrient levels are low. There is still much to learn about tritrophic interactions among insect herbivores, their host trees, and the ectomycorrhizal fungi of the trees. The potential benefit that ectomycorrhizae may have in terms of increasing resistance of trees to insect herbivores makes this area of research worth pursuing.

Acknowledgments

We thank Ann Lynch (USDA Forest Service Research and Development [USFS R&D], Rocky Mountain Research Station [RMRS], Flagstaff, AZ) and Peter Price (Department of Biological Sciences, Northern Arizona University [NAU], Flagstaff) for helpful comments on the draft manuscript. We are especially grateful to Subject Editor Barbara Bentz (RMRS, Logan, UT), an anonymous reviewer, and Matt Ayres (Dartmouth College, Hanover, NH) for suggestions that vastly improved the paper. We are grateful to Jan Huntsberger and Jerry Snow (USFS R&D, RMRS, Flagstaff, AZ) for their valuable assistance in maintaining the Douglas-fir seedlings and the western spruce budworm culture, respectively. We also thank Catherine Gehring (Department of Biological Sciences, NAU) for advice on experimental methodology, and Thomas Huntsberger (Analytical Services Laboratory, NAU) for conducting the chemical analyses. Our nondiapausing western spruce budworm colony was started with egg masses obtained from the Canadian Forest Service, Forest Pest Management Institute, Sault St. Marie, Ontario. This research was supported by research joint venture agreement No. 00-JV-11221605-178 between the RMRS (K.M.C.) and NAU (G.W.K.). This paper is part of a thesis submitted in partial fulfillment of the M.S. degree requirements at NAU by B.L.P.

References Cited

Bethlenfalvay, G. J., and S. Dakessian. 1984. Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in northern Nevada. J. Range Mgmt. 37: 312–316.

Castellano, M. A., and J. M. Trappe. 1985. Ectomycorrhial formation and plantation performance of Douglas-fir nursery stock inoculated with Rhizopogon spores. Can. J. For. Res. 15: 613–617.

Chen, Z., T. E. Kolb, and K. M. Clancy. 2001a. Mechanisms of Douglas-fir resistance to western spruce budworm defoliation: bud burst phenology, photosynthetic compensation and growth rate. Tree Physiology 21: 1159–1169.

Chen, Z., T. E. Kolb, K. M. Clancy, V. D. Hipkins, and L. E. DeWald. 2001b. Alozyme variation in interior Douglas-fir: association with growth and resistance to western spruce budworm herbivory. Can. J. For. Res. 31: 1691–1700.

Chen, Z., T. E. Kolb, and K. M. Clancy. 2002a. Effects of artificial and western spruce budworm (Lepidoptera: Tortricidae) defoliation on growth and biomass allocation of Douglas-fir seedlings. J. Econ. Entomol. 95: 587–594.

Chen, Z., T. E. Kolb, and K. M. Clancy. 2002b. The role of monoterpenes in resistance of Douglas fir to western spruce budworm defoliation. J. Chem. Ecol. 28: 897–920.

Chen, Z., K. M. Clancy, and T. E. Kolb. 2003. Variation in budburst phenology of Douglas-fir related to western spruce budworm (Lepidoptera: Tortricidae) fitness. J. Econ. Entomol. 96: 377–387.

Clancy, K. M. 1991. Douglas-fir nutrients and terpenes as potenital factors influencing western spruce budworm defoliation, pp. 124–134. *In* Y. N. Baranchikov, W. J. Mattson, F. Pain, and T. L. Payne [eds.]. Forest insect guilds: patterns of interaction with host trees. U.S. Dep. Agric. For. Serv. Gen. Tech. Pap. NE-153.

Clancy, K. M. 1992a. Response of western spruce budworm (Lepidoptera: Tortricidae) to increased nitrogen in artificial diets. Environ. Entomol. 21: 331–344.

Clancy, K. M. 1992b. The role of sugars in western spruce budworm nutritional ecology. Ecol. Entomol. 17: 189–197.

Clancy, K. M. 2001. Biochemical characteristics of Douglas-fir trees resistant to damage from the western spruce budworm: patterns from three populations, pp. 115–125. *In* R. I. Alfero, K. Day, S. Salem, K.S.S. Nair, H. Evans, A. Liebhold, F. Lieutier, M. Wagner, K. Futai, and K. Suzuki [eds.], Protection of world forests from insect pests: advances in research. IUFRO World Series volume 11. IUFRO Secretariat, Vienna, Austria.

Clancy, K. M. 2002. Mechanisms of resistance in trees to defoliators, pp. 77–101. *In* M. R. Wagner, K. M. Clancy, F. Lieutier, and T. D. Paine [eds.], Mechanisms and deployment of resistance in trees to insects. Kluwer Academic Publishers, The Netherlands.

Clancy, K. M., and R. M. King. 1993. Defining the western spruce budworm's nutritional niche with response surface methodology. Ecology 74: 442–454.

Clancy, K. M., J. K. Itami, and D. P. Huebner. 1993. Douglas-fir nutrients and terpenes: potential resistance factors to western spruce budworm defoliation. For. Sci. 39: 78–94.

Del Vecchio, T. A., C. A. Gehring, N. S. Cobb, and T. G. Whitham. 1993. Negative effects of scale insect herbivory on the ectomycorrhizae of juvenile pinyon pine. Ecology 74: 2297–2302.

Edmonds, R. L., J. K. Agee, and R. I. Gara. 2000. Forest health and protection. McGraw-Hill, Boston, MA.

Fellin, D. G., and J. E. Dewey. 1986. Western spruce budworm. U.S. Dep. Agric. For. Serv. For. Insect & Dis. Leafl. No. 53.

Fogel, R., and G. Hunt. 1983. Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. Can. J. For. Res. 13: 219–232.

Gehring, C. A., and T. G. Whitham. 1991. Herbivore-driven mycorrhizal mutualism in insect-susceptible pinyon pine. Nature (Lond.) 353: 556–557.

Gehring, C. A., and T. G. Whitham. 1994. Comparisons of ectomycorhizae on pinyon pines (*Pinus edulis;* Pinaceae) across extremes of soil type and herbivory. Am. J. Bot. 81: 1509–1516.

Gehring, C. A., and T. G. Whitham. 1995. Duration of herbivore removal and environmental stress affect the ectomycorrhizae of pinyon pines. Ecology 76: 2118–2123.

Gehring, C. A., and T. G. Whitham. 2002. Mycorrhiza-herbivore interactions: population and community consequences, pp. 295–320. *In* M.G.A. van der Heijden and I. R. Sanders [eds.], Mycorrhizal ecology. Springer Verlag, Berlin, Germany.

Gehring, C. A., N. S. Cobb, and T. G. Whitham. 1997. Three-way interactions among ectomycorrhizal mutualists, scale insects, and resistant and susceptible pinyon pines. Am. Nat. 149: 824–841.

Grove, T. S., and N. Malajczuk. 1994. The potential for management of ectomycorrhiza in forestry, pp. 201–210. *In* A. D. Robson and K. K. Abbott [eds.], Management of mycorrhizas in agriculture, horticulture and forestry. Kluwer Academic Publishers, The Netherlands.

Jones, C. G., and F. T. Last. 1991. Ectomycorrhizae and trees: implications for aboveground herbivory, pp. 65–103. *In* P. Barbosa, V. A. Krischik, and C. G. Jones [eds.], Microbial mediation of plant-herbivore interactions. Wiley, Inc., New York.

Kolb, T. E., K. A. Dodds, and K. M. Clancy. 1999. Effect of western spruce budworm defoliation on the physiology and growth of potted Douglas-fir seedlings. For. Sci. 45: 280–291.

Leyva, K. J., K. M. Clancy, and P. W. Price. 1995. Performance of wild versus laboratory populations of western spruce budworm (Lepidoptera: Tortricidae) feeding on Douglas-fir foliage. Environ. Entomol. 24: 80–87.

Marschner, H., and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159: 89–102.

Miller, O. K. 1982. Taxonomy of ecto- and endomycorrhizal fungi, pp. 91–101. *In* N. C. Schenck [ed.], Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, MN.

Muzika, R. M., and A. M. Liebhold. 2000. A critique of silvicultural approaches to managing defoliating insects in North America. Ag. For. Entomol. 2: 97–105.

Palermo, B. L., K. M. Clancy, and G. W. Koch. 2003. Feeding and oviposition behavior do not explain Douglas-fir resistance to defoliation by the western spruce budworm (Lepidoptera: Tortricidae). Environ. Entomol. (in press).

Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62: 244–251.

Schowalter, T., E. Hansen, R. Molina, and Y. Zhang. 1997. Integrating the ecological roles of phytophagous insects, plant pathogens, and mycorrhizae in managed forests, pp. 171–189. *In* K. A. Kohm, and J. F. Franklin [eds.], Creating a forestry for the 21st century: the science of ecosystem management, Island Press, Washington, D. C.

Shepherd, R. F. 1983. A technique to study phenological interactions between Douglas-fir buds and emerging second-instar western spruce budworm, pp. 17–20. *In* R. L. Talerico and M. Montgomery [eds.], Forest defoliator-host interactions: a comparison between gypsy moth and spruce budworms. U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. NE-85.

Van Sickle, G. A. 1987. Host responses, pp. 57–70. *In* Brookes, M. H., R. W. Campbell, J. J. Colbert, R. G. Mitchell, and R. W. Stark [eds.], Western spruce budworm, U.S. Dep. Agric. For. Ser. Tech. Bull. 1694.

Waring, G., and N. S. Cobb. 1992. The impact of plant stress on herbivore population dynamics, pp. 168–226. *In* E. Bernays [ed.], Insect-plant interactions, vol. 4. CRC, Boca Raton, FL.

White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia (Berl.). 63: 90–105.

Yanai, R. D., T. J. Fahey, and S. L. Miller. 1996. Efficiency of nutrient acquisition by fine roots and mycorrhizae, pp. 75–103. *In* W. K. Smith and T. M. Hinckley [eds.], Resource physiology of conifers. Academic, San Diego.

Received for publication 9 June 2002; accepted 18 February 2003.